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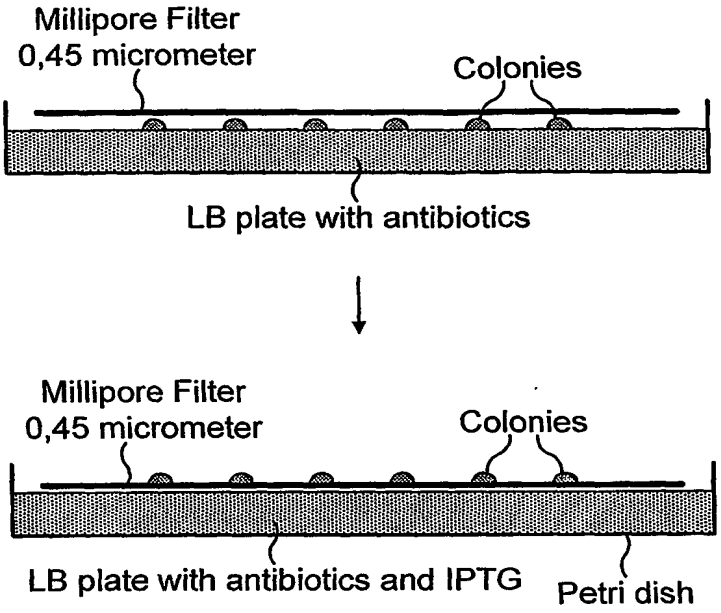



FIG. 1

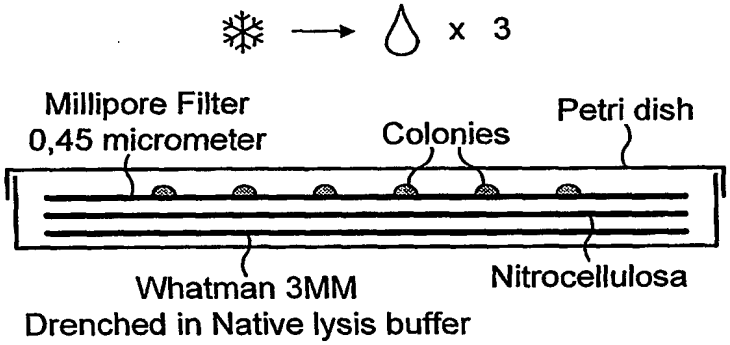



FIG. 2

BEST AVAILABLE COPY



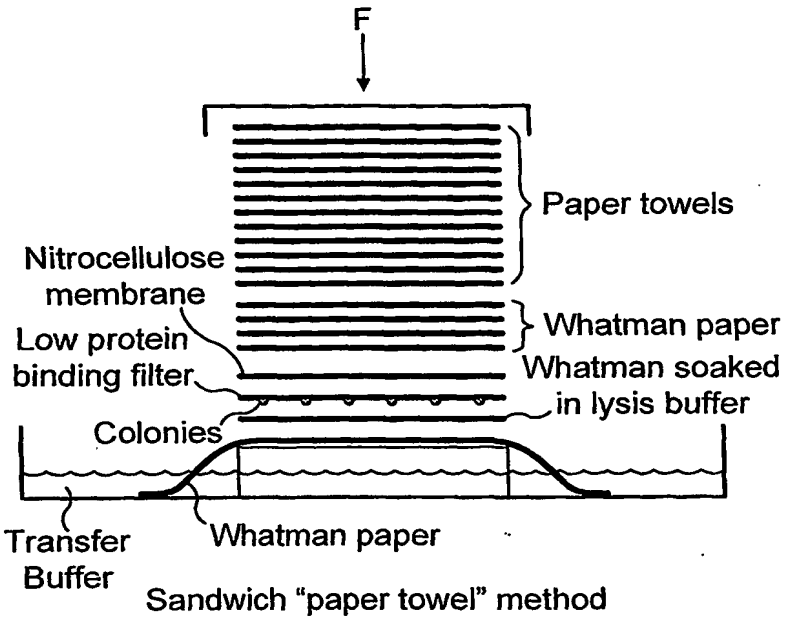



FIG. 3A

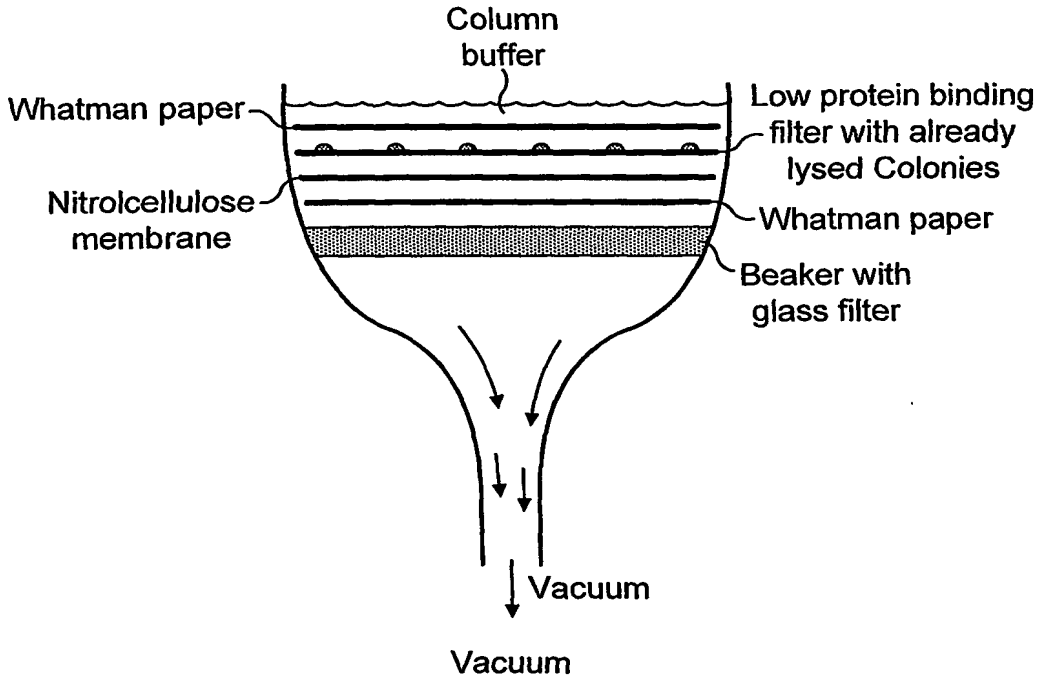



FIG. 3B

BEST AVAILABLE COPY



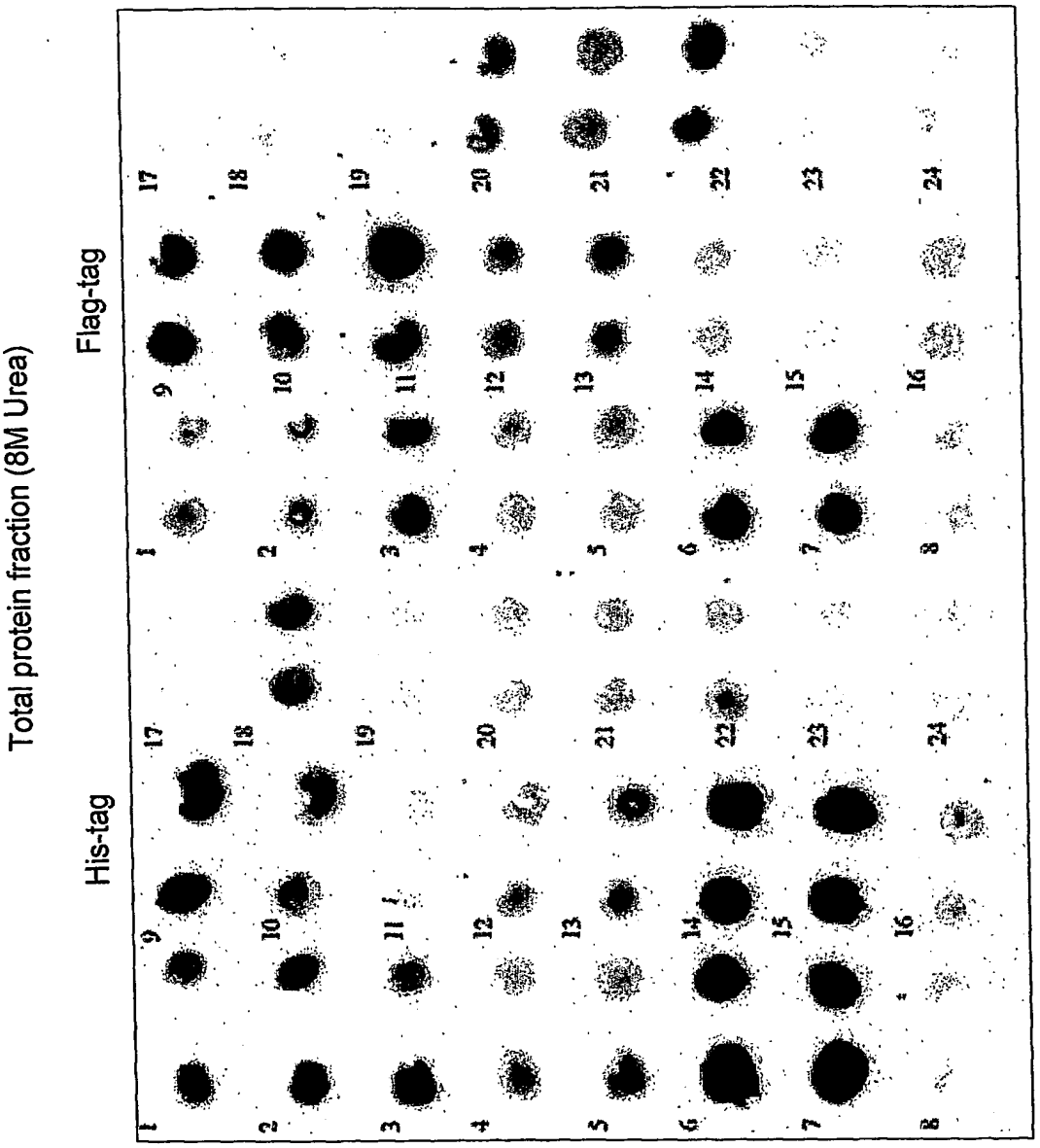



FIG. 4a

BEST AVAILABLE COPY



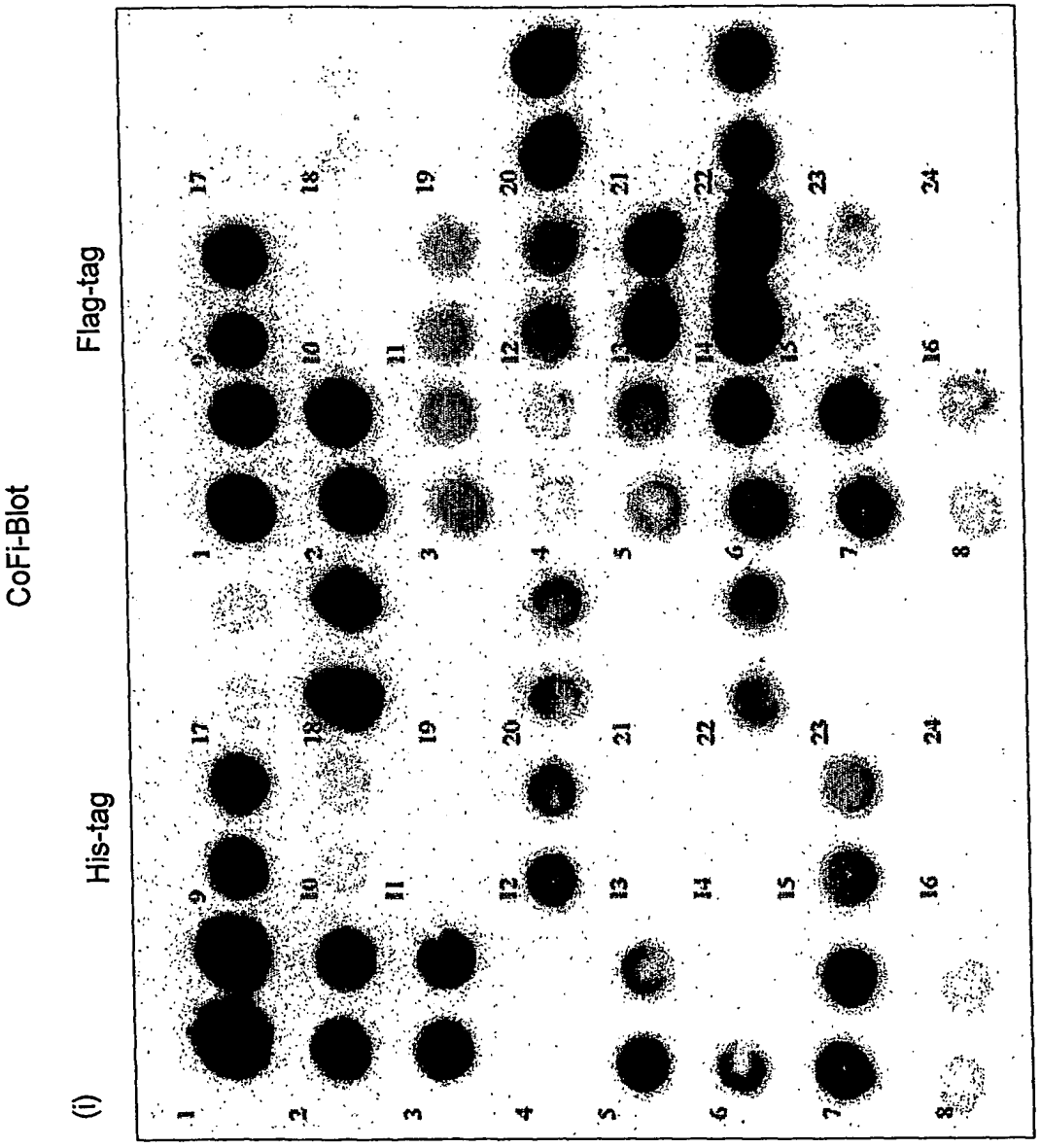



FIG. 4b

BEST AVAILABLE COPY



(ii)

| | CoFi-Blot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | His | | | | | | | |
| | | High | Low | High | Low | No | Medium | High | No |
| A | High | High | Low | High | High | High | High | High | No |
| B | High | Low | High | High | No | Medium | Low | High | No |
| C | High | No | No | Medium | Medium | Medium | Medium | Medium | No |
| D | No | Medium | Medium | Medium | Low | Medium | Medium | Medium | High |
| E | Medium | No | No | Medium | Medium | High | High | High | No |
| F | Low | No | Medium | High | High | High | High | High | High |
| G | High | Medium | No | High | Low | Low | Low | High | No |
| H | Low | No | No | Low | No | No | No | No | No |

False Positive or Negative

Predicted as soluble but in the most distant category

Predicted as soluble but in a neighbouring category

Correctly predicted in the right category

FIG. 4b CONT'D

BEST AVAILABLE COPY



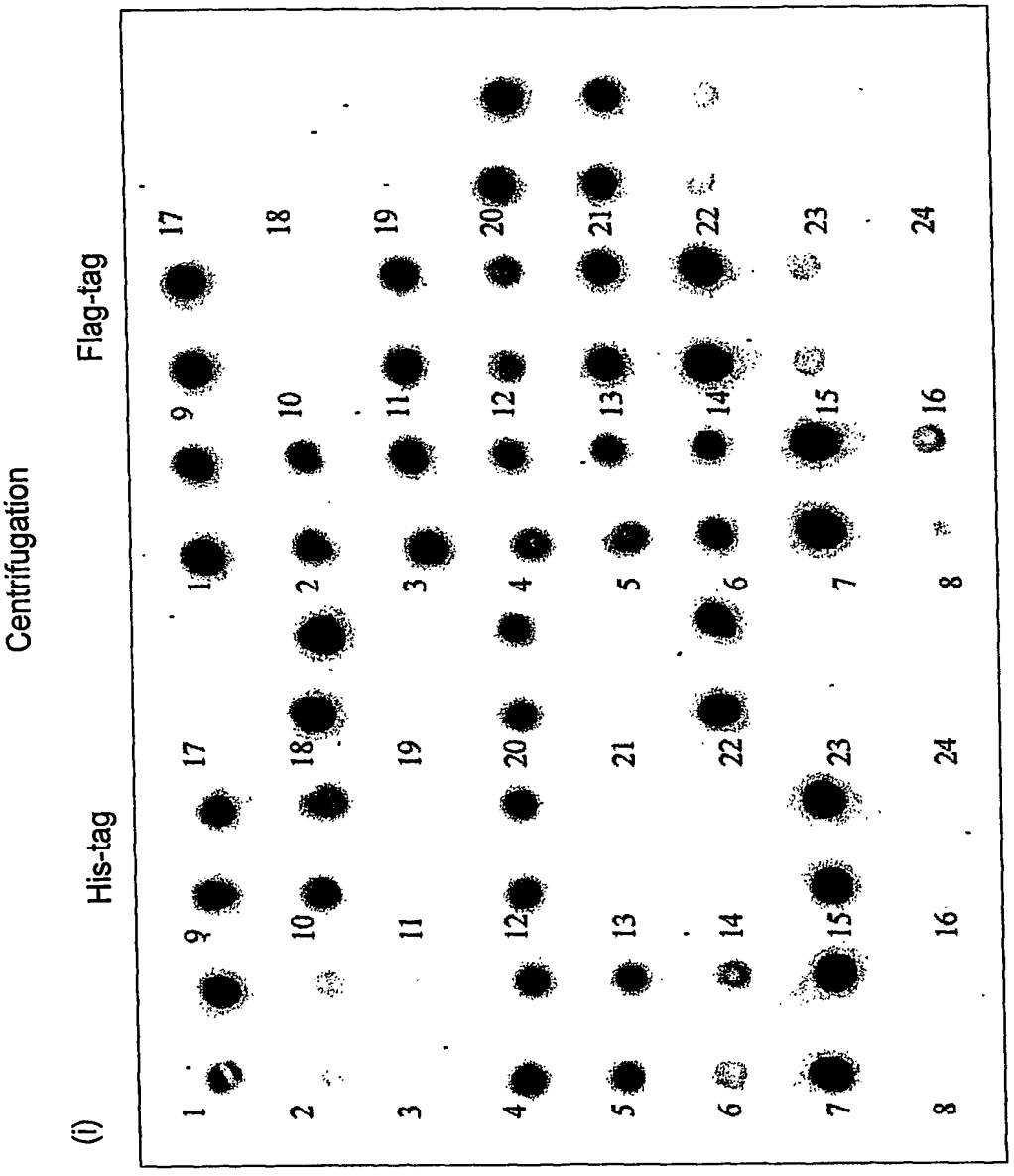



FIG. 4c

BEST AVAILABLE COPY



(ii)

| | Centrifugation | | | | | |
|---|---|---|---|---|---|---|
| | His | | | Flag | | |
| A | High | High | No | High | High | No |
| B | Low | Medium | High | Medium | No | No |
| C | Low | No | No | Medium | Medium | No |
| D | Medium | Medium | Medium | Medium | Low | High |
| E | Medium | No | No | Medium | High | High |
| F | Low | No | High | Medium | High | Low |
| G | High | High | No | High | Low | No |
| H | No | No | No | Low | No | No |

FIG. 4b CONT'D

BEST AVAILABLE COPY



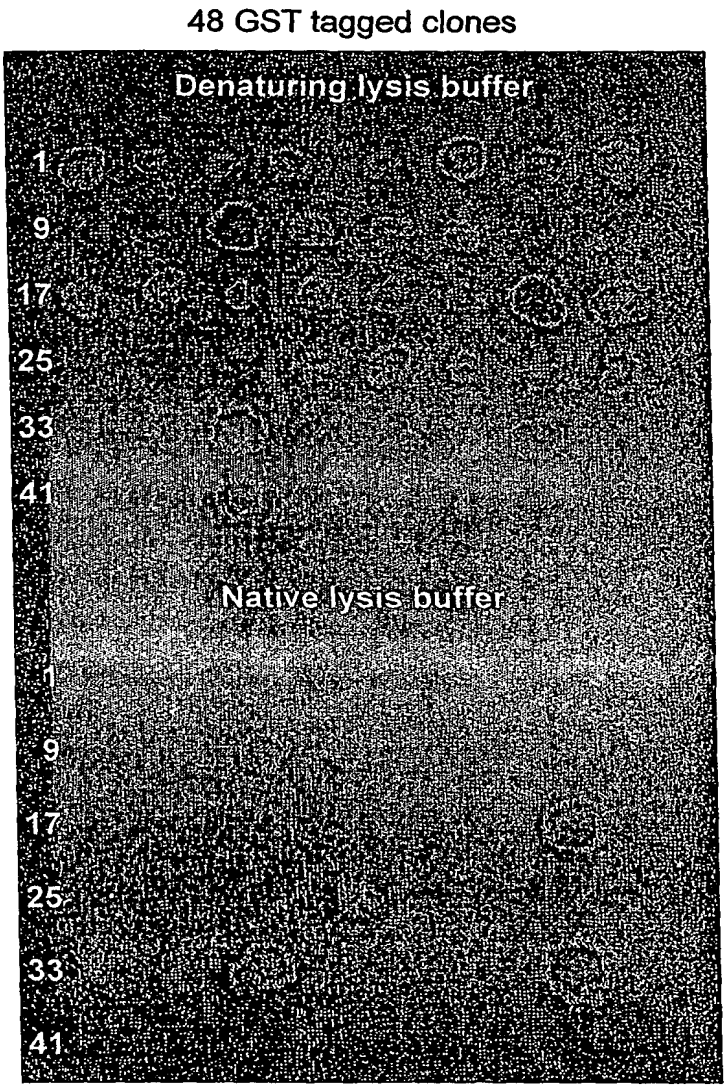



FIG. 5

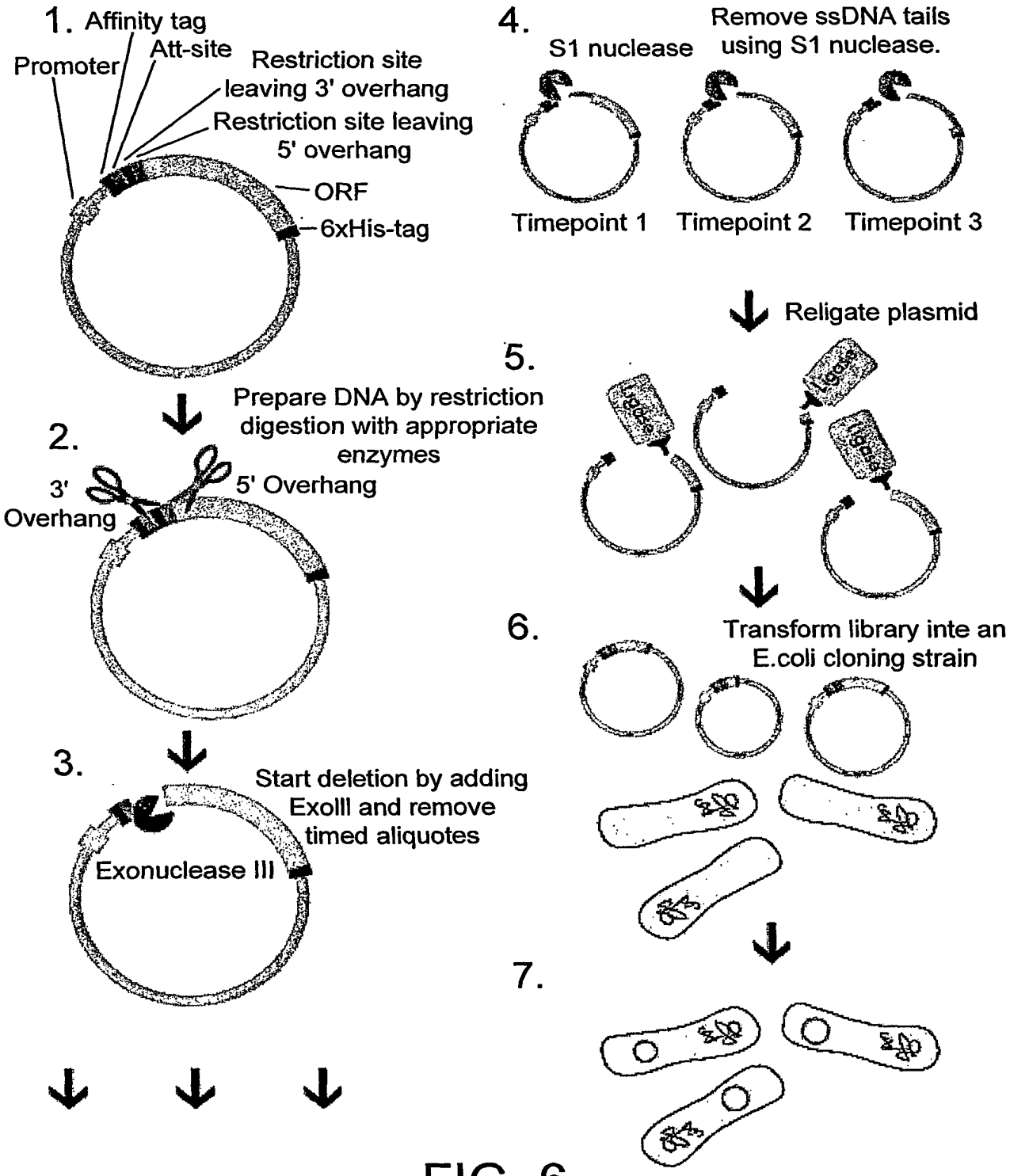
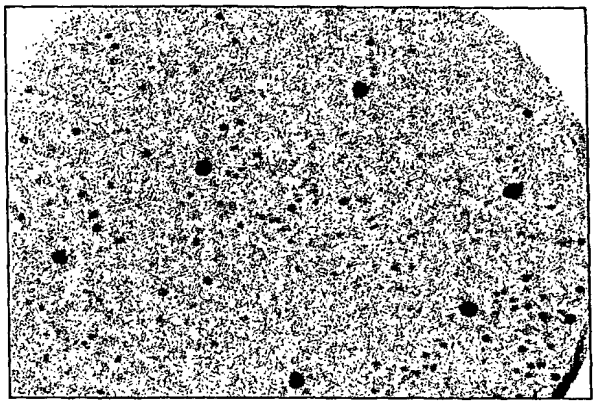
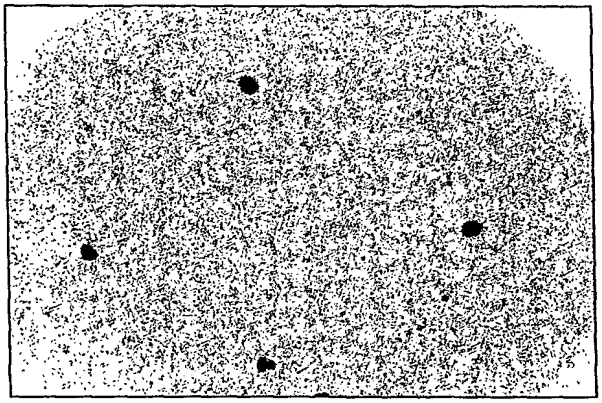

1. Affinity tag
Promoter
Att-site
Restriction site leaving 3' overhang
Restriction site leaving 5' overhang
ORF
6xHis-tag
Prepare DNA by restriction digestion with appropriate enzymes
2.
3' Overhang
5' Overhang
3.
Start deletion by adding ExoIII and remove timed aliquotes
Exonuclease III
4.
S1 nuclease
Remove ssDNA tails using S1 nuclease.
Timepoint 1
Timepoint 2
Timepoint 3
Religate plasmid
5.
6.
Transform library inte an E.coli cloning strain
7.

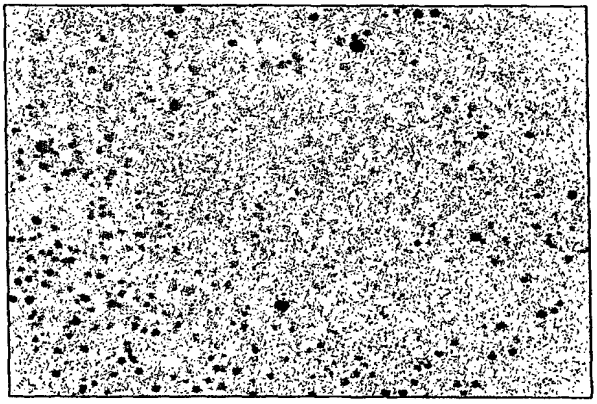
FIG. 6



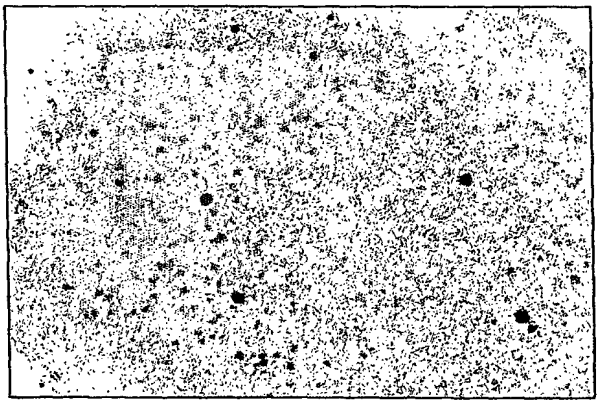
RORA 5-8 minutes
(~100-190 aa:s)



RORA 20-24 minutes
(~450-560 aa:s)



SOCS-2 1-4 minutes
(~0-90 aa:s)

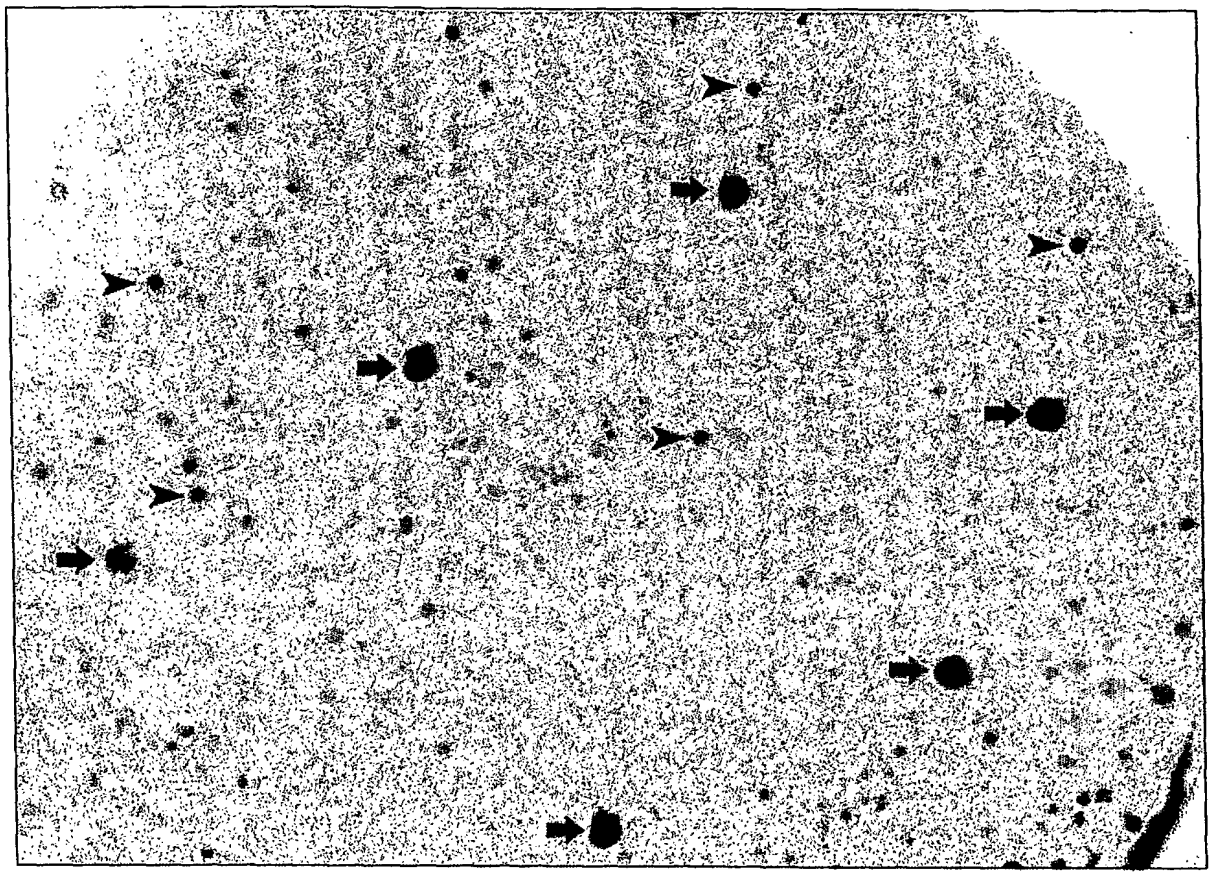


SOCS-2 9-12 minutes
(~210-300 aa:s)

FIG. 7a

BEST AVAILABLE COPY





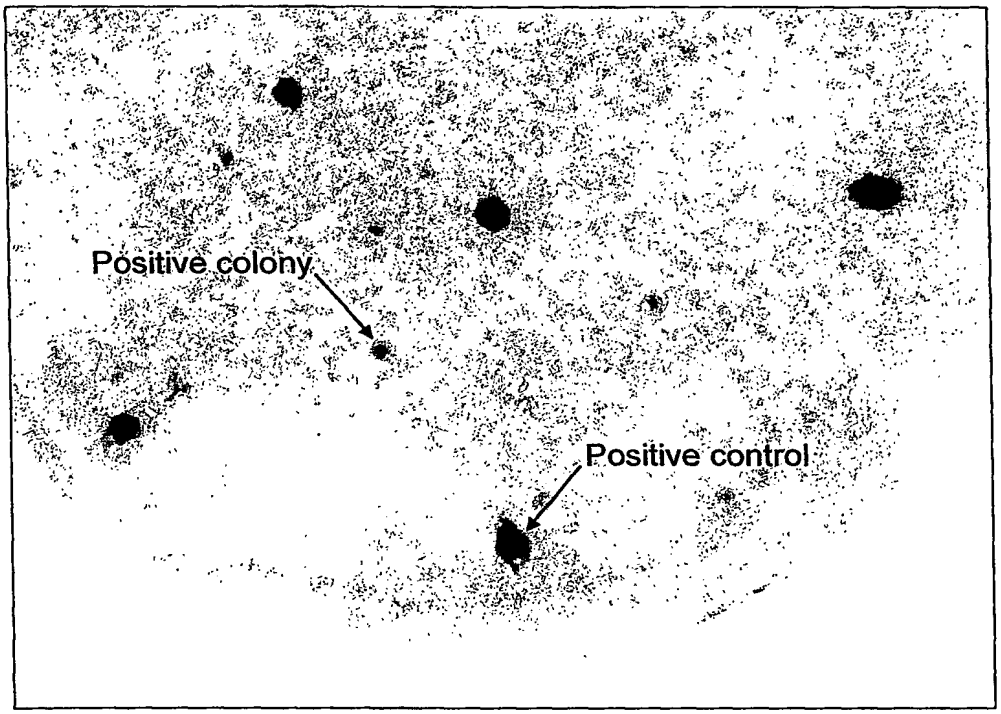
**Colony screen of a RORa library. The positive controls (➡) are added to the plate with the library to facilitate orientation. ➤ Indicates examples of colonies judged to produce soluble protein.**

FIG. 7b

BEST AVAILABLE COPY



Colony FiDo with reference




5:2500 Colonies

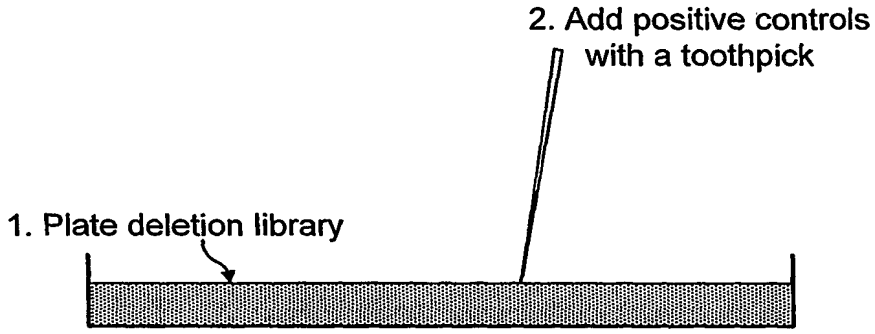



FIG. 8

BEST AVAILABLE COPY



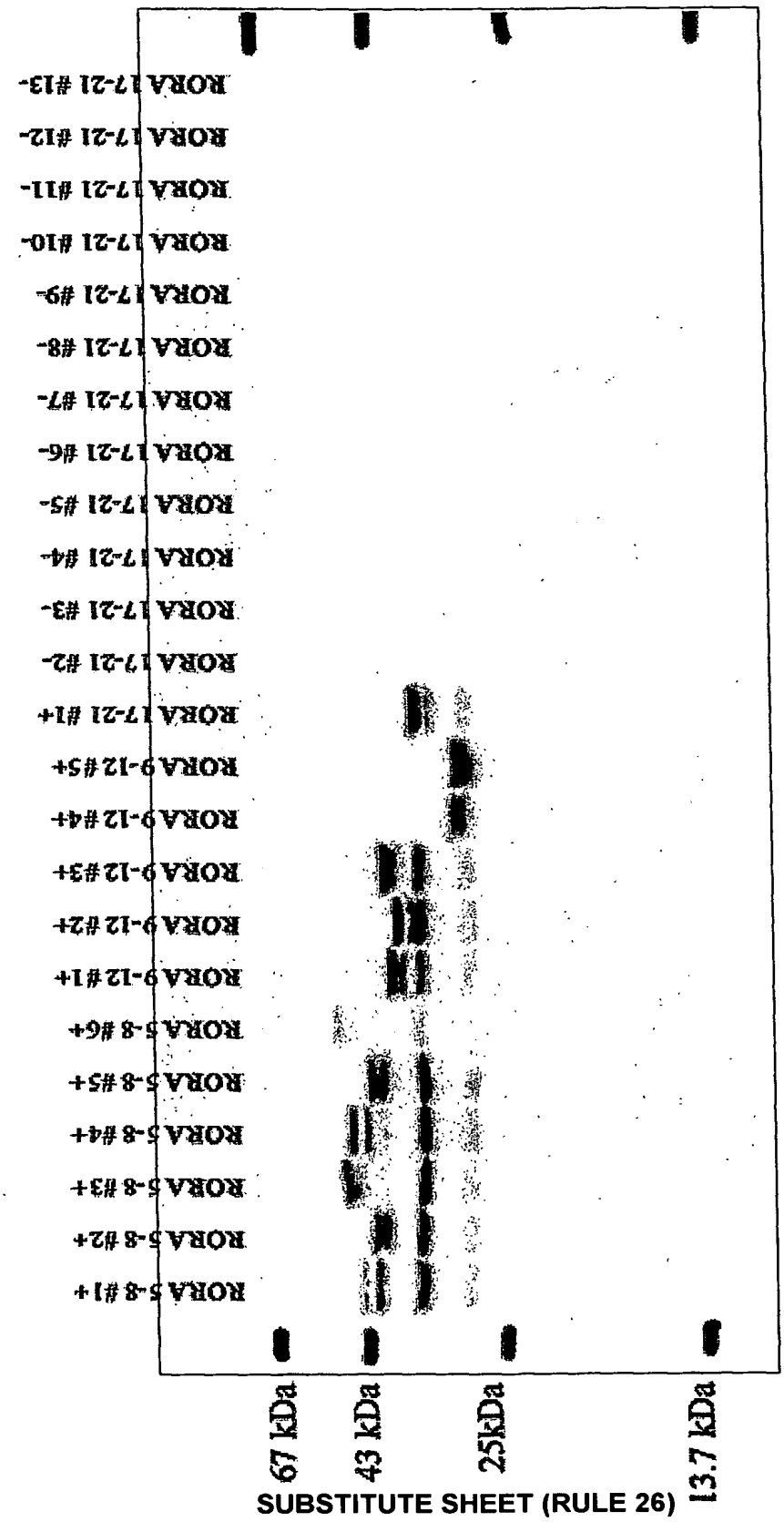



FIG. 9

BEST AVAILABLE COPY



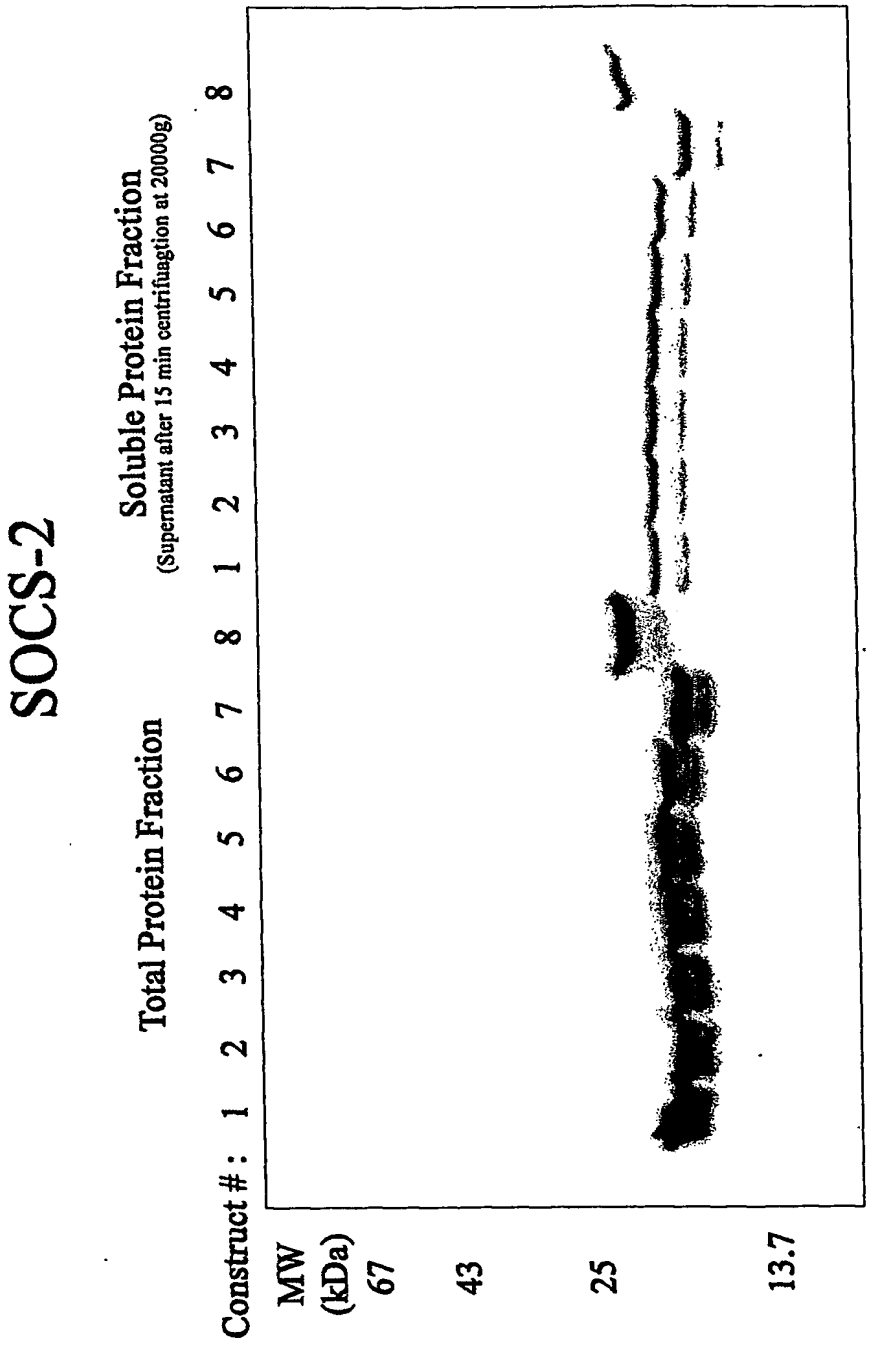



FIG. 10

BEST AVAILABLE COPY



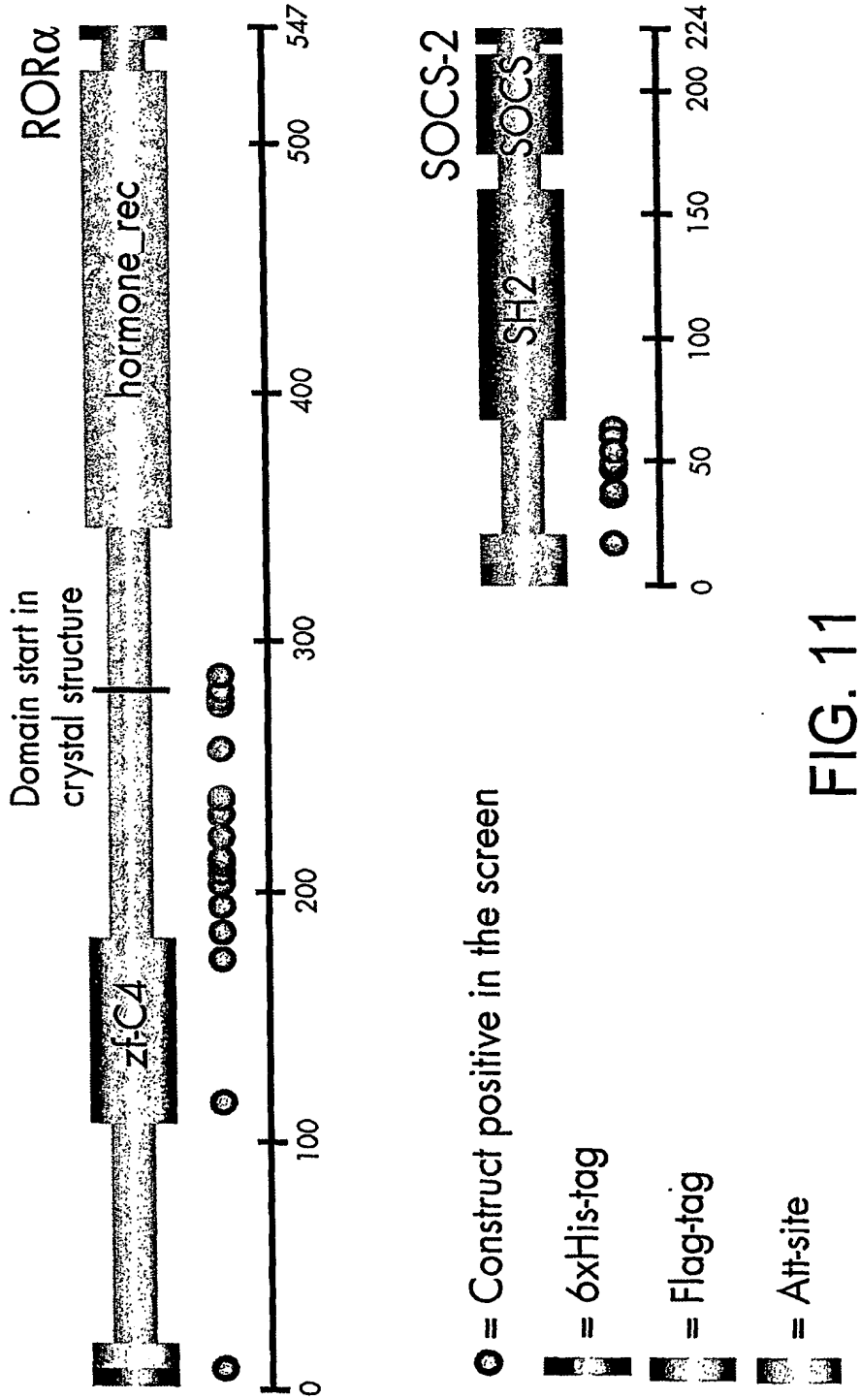



FIG. 11

BEST AVAILABLE COPY



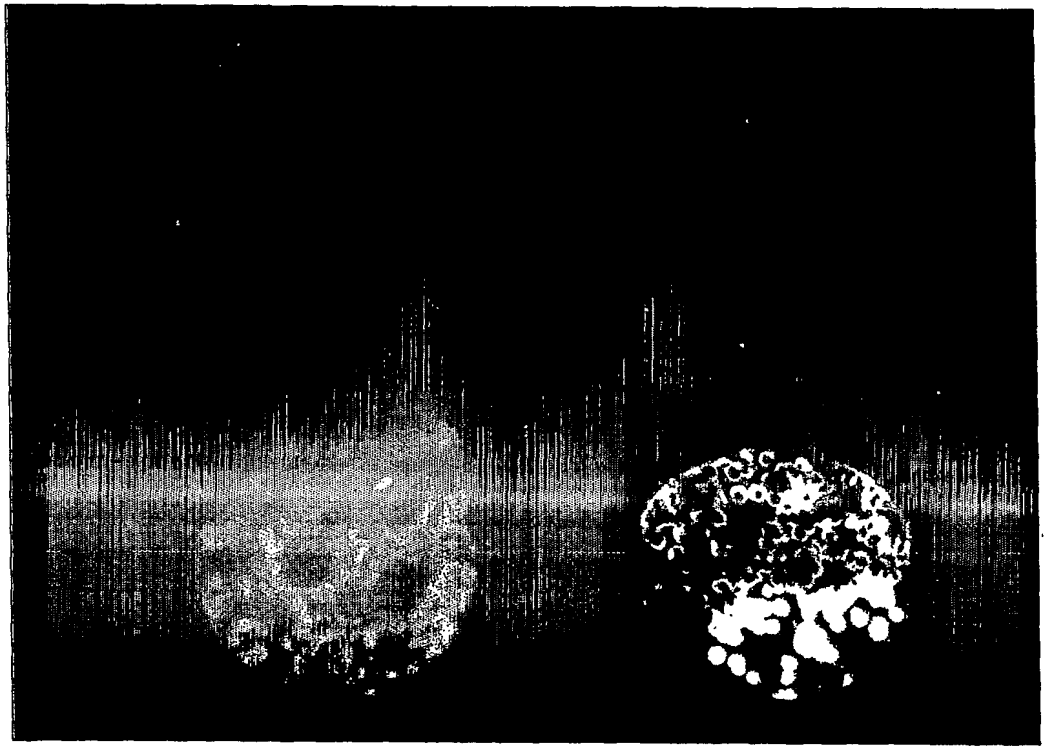


FIG. 12

BEST AVAILABLE COPY



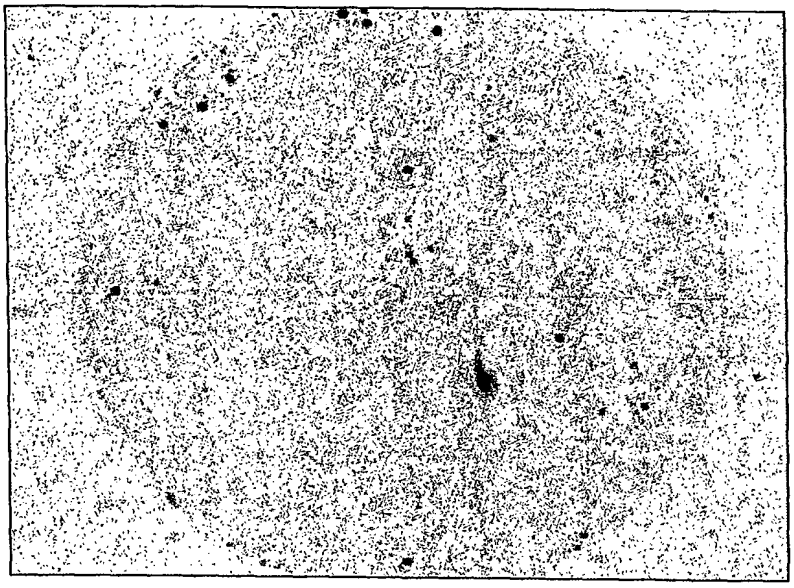


FIG. 13a

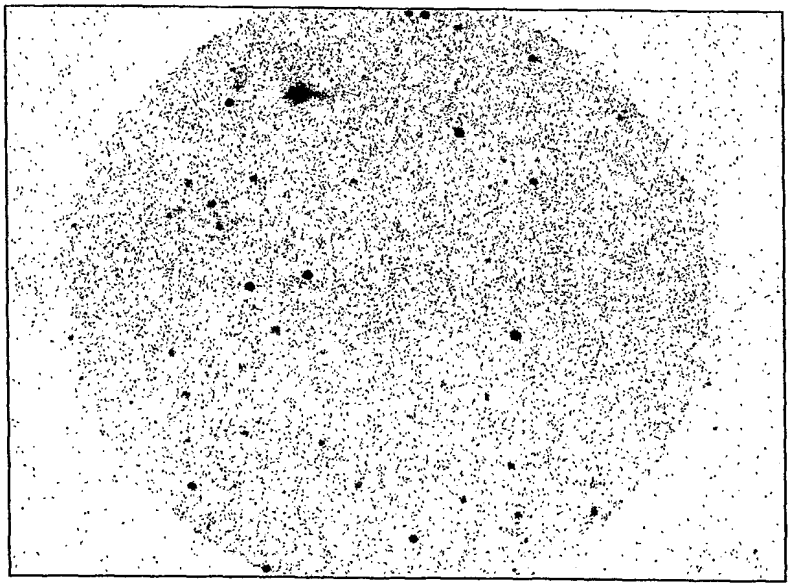


FIG. 13b

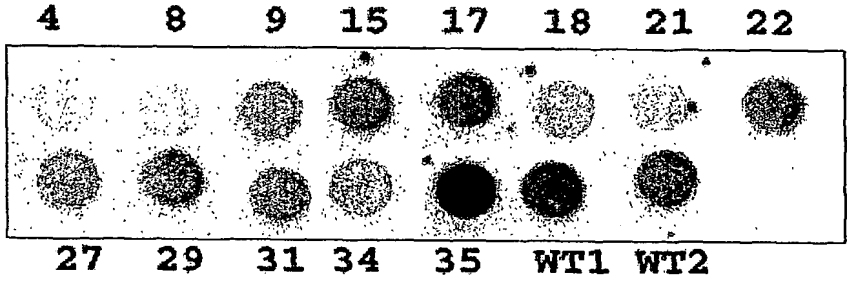



FIG. 14a

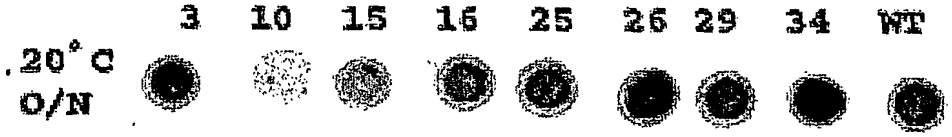



FIG. 14b